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The effect of glucose oxidase on reducing headspace oxygen in an airtight package

Meredith McHugh

THE EFFECT OF GLUCOSE OXIDASE ON REDUCING HEADSPACE OXYGEN IN AN AIRTIGHT PACKAGE

By

Meredith J. McHugh

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MASTER OF SCIENCE DEGREE

Fritz Yambach

David L. Olsson

Carol B. Whitlock

Christopher S. Penet

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I, Meredith J. McHugh, request that the Department of Packaging Science of Rochester Institute of Technology be contacted each time a request for reproduction is made

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ABSTRACT OF THESIS

THE EFFECT OF GLUCOSE OXIDASE
ON REDUCING HEADSPACE OXYGEN
IN AN AIRTIGHT PACKAGE

by Meredith J. McHugh

The objective of this study was to determine the effect of Glucose Oxidase in reducing headspace oxygen in an airtight package. Glucose Oxidase (0.05%) was included in the standard recipe for mayonnaise. A 0.10 ml of headspace gas was removed daily for 10 days from 15 jars of mayonnaise. Results indicated that headspace oxygen was reduced by the Glucose Oxidase. These results indicate that Glucose Oxidase may replace synthetic antioxidants used in food packaging currently. Studies may be continued to research the possibility of the reaction continuing after the product is opened to extend shelf life.

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INTRODUCTION

The American consumer is now accustomed to buying products that are microbiologically safe and remain high in organoleptic quality for extended periods of time. The shelf life of a food product depends on many factors: microbial growth, enzyme reactions and oxygen and water gain or loss. By far the most crucial factor is the rate at which oxygen enters the package (Russell, 1989, p. 92). Food processors have used various methods to extend shelf life by preventing oxidation of fats to extend markets and minimize economic loss.

Oxidation of Fats

Oxidation of fats is a frequent cause of poor shelf life and off-flavors in processed foods. Even during storage, fats can undergo oxidative changes that create objectionable flavors and odors. Oxidation of fats proceeds in three basic steps:

1. Initiation -- introduction of oxygen to fat,
2. Propagation -- generation of hydroperoxides and free radicals; and
3. Termination -- the combination of free radicals with each other or with other compounds and the formation of further peroxides and the release of oxygen back to the system. Hydroperoxides are the major products of fat oxidation. They can break

> down to secondary products or they can react with proteins and enzymes in the food products (Ory, 1985, p. 205).

Preventing this oxidation and extending shelf life has been a concern for thousands of years.

Early interest in increasing shelf life

Since primitive tribes gathered and stored foods to tide them over the winter, people have been looking for ways to extend the shelf life of food (Forcinio, 1994, p. 87). Various methods are used to extend the shelf life, but with the consumers' growing awareness of preservatives, food processors have been searching for natural alternatives for extending shelf life. Increased shelf life not only extends the time the product is on the shelf, but there are fewer returns and a greater distribution radius can be attained. Instead of limiting the distribution to just local markets, there is nationwide distribution. Some companies can export products only because they control the amount of oxygen in a package and the degradative reactions occurring from oxygen reactions (Heganbart, 1994, p.80).

OBJECTIVE OF THE STUDY

The objective of this study is to determine the feasibility of applying the enzyme system, glucose oxidase-catalase, to reduce headspace oxygen in a mayonnaise packaging system.

EXTENDING SHELF LIFE OF PERISHABLE PRODUCTS BY MODIFYING ENVIRONMENTAL FACTORS

External Environments

By placing the product/package system in a controlled enviroment, shelf life can be extended by limiting degradative reactions.

Refrigeration

Before mechanical refrigeration systems were introduced, ancient peoples cooled their food with ice transported from mountains. Wealthy families used ice cellars--pits dug into the ground and insulated with wood and straw to store the ice. Using these pits, packed snow and ice could be preserved for months. Ice was the principle means of refrigeration until the beginning of the 20th century (Landis, 1993).

Today most refrigerators use vapor compression systems. A refrigerant (vaporized fluid) passes through chiller plates in the freezer section that contains coils. The refrigerant is partially evaporated and then continues to the main refrigeration compartment. Small fans circulate the cold air throughout the compartment.

Refrigeration keeps food from spoiling because bacterial growth is slowed at lower temperatures. A typical refrigerator is kept at 40° F. A temperature which will prevent food spoilage for days. Foods kept frozen at temperatures near 0° F can be stored for months without decay or loss of flavor.

On the other hand, refrigeration causes several problems in the preservation of foods. Some problems are:

1. eventual loss of quality,
2. dehydration of food products,
3. cell destruction of fresh produce at low temperatures,
4. expense of refrigeration in entire distribution system,
5. lack of exact temperature control during distribution and;
6. lack of refrigeration capability in all countries.

Controlled Atmosphere Storage

Controlled atmosphere storage for some products reduces spoilage. Desired atmospheric conditions are maintained at specific levels over time by controlling the gases which are present during storage. The gases that are controlled are oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2) and sometimes ethleyne. Controlled atmosphere has offered a potential shelf life increase of over 400% for many products. Thus, many products can be distributed over longer distances or with fewer deliveries (Hotchkiss, 1989, p. 41).

Controlling the gaseous environment is not a new idea. In the 19th century, scientists discovered that the elevation of carbon dioxide and a decrease in oxygen retarded respiration in some foods and slowed the growth of aerobic microorganisms. Research information on the use of controlled atmosphere for extending the shelf-life of fruit, vegetables, and meats was done during the 1920's. By 1938, 26% of chilled carcasses shipped from Australia were shipped under a carbon-dioxide-enriched atmosphere.

Controlled atmosphere storage of produce has given the consumer a wide variety of fruits and vegetables during the off season that were unavailable just a few years ago. Wegman's, a Rochester based grocery store chain, currently has large chambers in their distribution center for ripening bananas. Wegman's receives the bananas when they are still green and uses the chambers with controlled levels of ethylene, a gas that is given off by produce when it is ripening. Increasing ethylene will cause bananas to ripen faster and removing of ethylene produced by the fruit will slow ripening down. This process allows for ripening according to market demand.

Many studies have been completed on the effect of apples stored at low levels of CO_2. Controlled atmosphere storage of 'Delicious' apples at variable carbon dioxide concentrations was completed by Drake, Eisele and Waelti (1993). This study demonstrated that for two strains of

Delicious apples held in controlled atmosphere for nine months, the mean values for firmness, external and internal color, and titratable acidity did not differ between the apples held in different controlled atmosphere conditions. Sensory panelists found no flavor differences in apple juice after long term controlled atmosphere storage regardless of CO_2 storage level.

One must consider the high cost of equipment, installation, and the removal of gases during long term controlled atmosphere before implementing this system (Drake et al., 1993). High cost may prohibit the use of this process.

INTERNAL ENVIRONMENTS

Another approach is to use a package to construct a barrier to enviromental conditions and create a separate internal environment.

Modified atmosphere packaging

Modified atmosphere packaging uses the same principles as controlled atmosphere storage, altering gases surrounding the product. It may be easier to control the small space surrounding the product than the whole environment as with controlled atmosphere. Modified atmosphere packaging extends shelf life by reducing respiration rates while at the same time avoiding anaerobic processes that lead to adverse changes in texture, flavor and aroma. Early attempts at modified atmosphere packaging were unsuccessful due to the limitations of packaging materials available. The materials were unable to hold the proper percentage of gases within the package. With the increase in packaging technology, packaging developers are now able to use the modified atmosphere packaging technology successfully. The growth of modified atmosphere packaging can be attributed to the following:

1. Development of packaging materials with excellent water vapor and gas barriers.
2. Market needs and consumer demands for convenience--consumers are willing to pay for fresh foods with minimum preparation time (ex. the growth of prepared vegetables).
3. modified atmosphere packaging uses 18-20% less energy than freezing
4. modified atmosphere packaging provides a high quality product; and
5. Products can be distributed over longer distances and with fewer deliveries (distribution costs are decreased) (Farber, 1991).

The gases used in modified atmosphere packaging are gases that are present in ambient air. The gases most often used are:

1. Oxygen

- Maintains the "bloom" color of fresh meat
- Sustains basic metabolism of fruits and vegetables which respire
- Prevents anaerobic bacteria such as C. botulinum

2. Carbon Dioxide

- Inhibits bacteria and mold growth
- Prevents insect growth

3. Nitrogen

- Prevents package collapse when used as a filler in products that absorb CO_2
- Chemically inert
- Prevents oxidation, rancidity, mold growth and insect attack (Inns, 1987).

Two Categories of modified atmosphere packaging

Passive modification -- The atmosphere in the package is modified as a result of the consumption of oxygen and the generation of carbon dioxide by the product. For passive modification to be effective, the packaging material used must allow oxygen to enter the package at a rate usually lower than the consumption of oxygen by the product. At the same time it must allow carbon dioxide to be vented from the package. Failure to achieve this rate will result in decreased oxygen and increased CO_2 within the package causing spoilage.

Active modification -- The atmosphere of the package is physically changed by the food processor. Types of active modification include:

1. Vacuum packaging: Products are placed in a film of low oxygen permeability and the air is removed from the package by vacuum. The oxygen can be reduced to less than 1%. Carbon dioxide is produced by the tissue increasing the CO_2 to 10-20% therefore extending shelf life.
2. Modified atmosphere gas packaging: This process manually injects the appropriate gas mixture.

For these methods to be successful, packaging materials must have a high oxygen barrier. If proper packaging materials are not used, gases will escape causing food spoilage (Hui,1992).

Types of packaging equipment for modified atmosphere gas packaging

1. Continuous forming -- makes a tube of film that encloses the product; an appropriate gas mixture is introduced in a continuous flow into the package to dilute the air present; the ends of the package are then sealed.
2. Thermoforming -- product is placed into a formed tray and a vacuum is used to remove the air, the vacuum is broken by an appropriate gas mixture and the package is heat sealed.

Disadvantages to modified atmosphere packaging

1. Initial high cost of packaging equipment and training
2. Discoloration of red meat pigments
3. Leakage of gases used to control atmosphere causing spoilage
4. Fermentation and swelling of certain foods
5. Strict sanitary environment required
6. Temperature control necessary
7. Potential growth of anaerobic microorganisms of public health significance.

Microbiological hazards safety concerns have prevented the growth of this process. The U.S. Food and Drug Administration is quite concerned that this new technology may not be safe and may increase the risk of pathogenic bacteria developing in foods. Currently, the data base is not sufficiently large to make a decision. A major goal is to increase this data base so that decisions can be made with greater confidence. If one had more confidence in the safety of modified atmosphere packaging, one might make wider use of the technology (Hotchkiss, 1989).

The most important concern is that modified atmosphere packaging may inhibit spoilage indicator organisms while promoting pathogen development. When a food is temperature or otherwise abused, or the packaging system or package fails, four results are possible:

1. The numbers of both the pathogenic and spoilage organisms remain low. This is the least hazardous situation.
2. The spoilage organisms proliferate but the pathogens do not. While this is undesirable from the standpoint of food quality the hazard is not great, since the indication of spoilage (smell and texture) will prevent consumption.
3. Both the pathogenic and spoilage organisms proliferate. This means that if consumed, the food could be harmful.
4. Pathogenic organisms proliferate without the concurrent growth of the indicator spoilage organisms. In this case, there is no sensory warning that the food may be hazardous (Hotchkiss, 1989, p.42).

USING BARRIER PACKAGING MATERIALS TO EXTEND SHELF LIFE

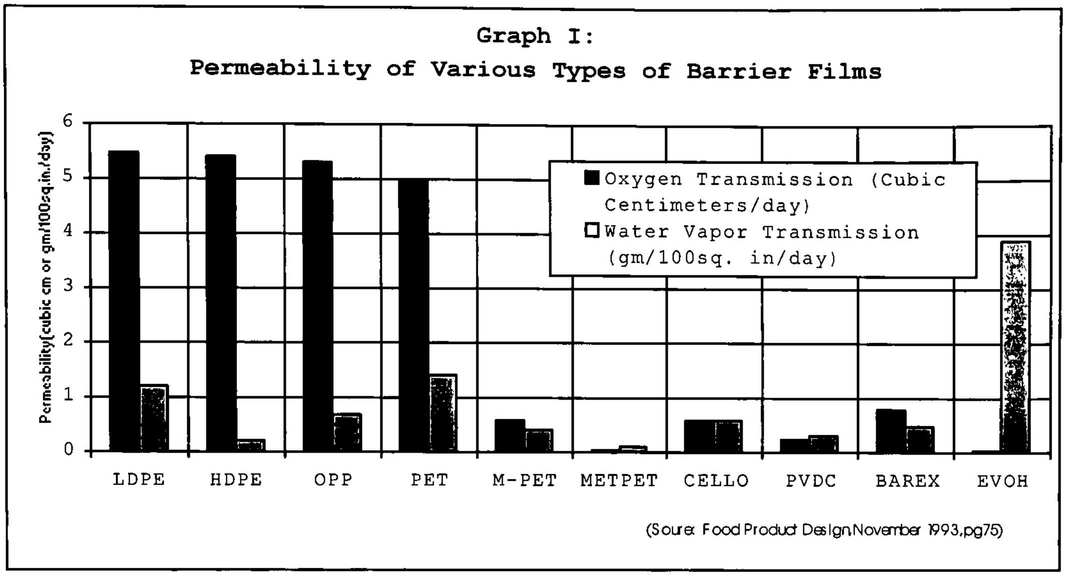
Barrier materials are usually classified as materials that protect a product through the end of its shelf life by reducing ingress of deterioative materials. High barrier packaging protects products from:

1. Ingress of oxygen.
2. Loss of carbonation.
3. Loss or gain of moisture.
4. Contamination.
5. Permeation of solvents, odors, and flavors.

Until the early 1970s, the only packaging materials that would provide this type of protection were glass, metal and Polyvinylidene chloride (PVDC).

Packaging technology has come a long way, and high barrier plastics provide a new dimension for food packagers. With improvements in an area of plastics known as barrier polymers, many plastic packages now rival the excellent shelf life and barrier properties that glass and metal have always provided food packagers. (See graph I)

Although there are numerous polymers available, ethylene vinyl alcohol copolymer (EVOH) is one high barrier material which has contributed to the acceptance and functionality of plastic packaging for food products. EVOH resins are produced by copolymerizing ethylene and vinyl acetate and then hydrolyzing the vinyl acetate to vinyl alcohol. The resulting polymer offers superior barrier properties to gases, solvents, odors, and aromas, together



with excellent processability (Schaper, 1991). Although EVOH provides excellent barrier properties against oxygen permeation, it is not highly effective in preventing water vapor transmission. On the other hand, a film like high density polyethylene (HDPE) provides protection for water vapor transmission but performs poorly as a barrier for oxygen permeation. As a result, packaging suppliers have created multi-layered films that combine the optimum barrier properties of different types of packaging films. These multilayers are created by co-extrusion and can provide a cost efficient barrier package.

Growth of Barrier Technology

Growth of these multi-layered structures has been extraordinary. In late 1983, the first multilayer high barrier plastic bottle was introduced by American National

Can. The bottle, called the "gamma" bottle, had a structure consisting of:
polypropolyne/Regrind/Tie/EVOH/Tie/Polypropylene, and was used to package Wishbone Bar-B-Que sauce. This led to numerous uses of multilayered structures in the next 5 years (See Table 1). The popularity of these structures shows the consumer's willingness to pay for conveniences offered by the high barrier plastic containers. Conveniences such as, squeezability, shelf stable, single serve size, shatterproofness and microwavability.

TABLE 1- Multilayer Package Introductions

Year	Bottles	Formed	Flexible	Coex Coating	Tubes	Total
1983	2	...	1	...	...	3
1984	6	...	4	...	..	10
1985	30	5	2	...	...	37
1986	41	12	9	...	4	66
1987	54	10	3	20	7	94
1988 (thru June)	60	32	17	8	3	120
Total	165	44	27	23	11	270

(Source: Schaper 1991 p36)

Enviromental Issues Surrounding Barrier Materials

Plastic packages providing superior barrier protection for food products are complex structures. Are these packages being over-layered and have they become too complex? "Yes" and "no" industry spokesmen say. There is always a trade off between long shelf life and a complicated package versus a shorter shelf life and a less complicated package. For example material used in the first structure might be polyethylene terepthalate (PET) which alone cannot be called a high barrier material. PET is often used as a barrier and structural component. PET is not an effective and economical barrier for many products such as mayonnaise since the container thickness may reach 30 mil. The result is a high cost container and takes a long time to make and fill. A high barrier material such as PVDC or EVOH at one mil thickness would be more economical and work more effectively (Russell, 1989, p. 98).

It would be an ideal situation to return to monolayered packages, but are consumers willing to give up convenience and the fresh foods without preservatives that are packaged in these high barrier, multilayered packages? The more complex the package, the more difficult it is to recycle. The layers have to be separated and then reground, which is a very costly operation. To return to a monolayer structure for plastics, a very sophisticated and effective resin must be developed.

Other environmental issues surrounding high barrier film is that many of these films contain chlorine - the primary culprit responsible for the depletion of the ozone layer of the earth's stratosphere. Intense environmental pressures surrounding chlorine-containing barrier resins are pushing for the development of new films.

A relatively new film that can satisfy all the above needs is a glass-coated flexible barrier film, that is prepared by depositing inorganic glassy materials (for example, silicon dioxide or aluminum oxide) on a smooth plastic substrate. Glass-coated plastics are microwavable, retortable and recyclable. These coatings are not sensitive to moisture, are an excellent barrier to moisture and oxygen and are applied in small amounts to the base film. Glass-coated films are reported to have the barrier properties similar to those of aluminum foil but are microwavable. The amount of actual glass (silicon dioxide) in these films is so small that it can be essentially ignored when these materials are recycled. Thus only the base material must be considered in potential recycling operations (Marsili, 1993 p70). Some problems have been reported with these glass films. The material is hard to score, therefore, making it difficult to form on packaging machinery. Off odors and flavors have been reported in juices packaged with these materials.

EXTENDING SHELF LIFE OF PERISHABLE PRODUCTS BY USE OF SCAVENGING SYSTEMS

Scavenging systems reduce or supply gases that may reduce quality of packaged food products such as oxygen, carbon dioxide, ethylene and water vapors.

Oxygen absorbents

Sachets containing chemicals that absorb oxygen are placed in the package. Oxygen absorbers can routinely maintain a .01 percent level of oxygen (Spaulding 1988). Oxygen absorbers are being used with a multitude of food items in Japan, including nuts, cheese, coffee, processed meats, cakes, confection and dried fruit. The combination of an oxygen-barrier and oxygen absorber packet can reduce unwanted changes in food due to the presence of oxygen. The packet usually contains powdered, active iron-oxide which chemically absorbs the free oxygen in the package. The level of oxygen is reduced to .01 percent or less within one day at room temperature. Due to this low residual-oxygen level, another bonus is that mold cannot grow on the food. Oxidative color and flavor changes are also eliminated. With an oxygen scavenger sized for excess capacity, whatever oxygen permeates the barrier material would continue to be absorbed.

Problems with using sachets:

1. The USDA and the FDA have established no firm regulations for the packet's direct contact, however they have released opinions and guidelines for an absorber's use in non-contact and dry product applications.
2. The possibility of accidental ingestion of the packet by a consumer, especially young children. While regulations require that the contents be non-toxic, the concern remains that if someone were to ingest the contents mistakenly thinking they were a seasoning of some sort, the flavor

would reflect poorly on the product (Hegenbart, 1994).

3. Consumers in the United States are thought to be suspicious of anything found in the food package besides food.
4. Proper sealing must be achieved. Incomplete seals, holes, and other means of permeation will either negate the desired effects or drive up costs because a more powerful sachet is required to maintain control.
5. Reducing oxygen to such low levels may enhance the risk for growth and toxin production of pathogenic bacteria. Because of this, foods packaged with oxygen scavengers should be carefully examined for microbial growth, especially when use is not limited to low water activity and low pH products that inhibit these bacteria (Powers, Berkowitz, 1990 p.767).

Overcoming these problems leads to other possibilities. One could create a package with a specially formed cavity to hold the oxygen absorber or incorporate the material into a pressure-sensitive label that can be attached using labeling equipment. The oxygen absorbing chemicals can be incorporated into packaging by blending with materials such as PVC crown cap liners. Lower oxygen levels in the headspace of bottles extend the shelf-life of beer. Tests show that even after one month, beer capped with the oxygen absorbing crowns and stored at room temperature tastes significantly better than beer capped with conventional crowns and stored under the same conditions (Marsili, 1993 p.69). Having achieved success with bottled beer applications, this technology is now being tested for foods in glass jars. Some other technologies concerning placing scavenging systems in materials are being researched. Incorporating silicate or aluminum oxide mixtures into packaging film polymers to see if they can absorb excess carbon dioxide for respiring fruit and vegetable that are sensitive to excess carbon dioxide is being researched. Removal of ethylene gas, a natural metabolic process of many fresh produce items, by reaction with potassium permanganate can prolong the shelf-life and can be blended into polymer films.

EXTENDING THE SHELF LIFE OF PERISHABLE PRODUCTS BY SYNTHETIC ANTIOXIDANTS.

Antioxidants neutralize free radicals to interfere with their destructive effects. Their ability to do this is based mainly on their phenolic structure. Antioxidants function by donating a hydrogen atom to the fat-free radical to reform the fat molecule, or by donating a hydrogen to a peroxide-free radical to form a hydroperoxide and a stable antioxidant free radical.

Hundreds of synthetic antioxidants have been evaluated for antioxidant effectiveness. Of these, only four synthetic antioxidants have found widespread use: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiarybutyl hydroquinone (TBHQ). (Dorko, 1994 p. 33)

1. BHA is a mixture of two isomers, 2 tertiarybutyl-4-hydroxyansisole and 3 tertiarybutyl-4-hydroxyansisole. The 3-isomer is the better antioxidant. Commercially produced BHA typically contains at least 90% of the 3-isomer. BHA, a white waxy solid, is effective in animal fats, but relatively ineffective in vegetable oils. It provides good carry through (abilities of antioxidant to be added to food, survive processing, and add stability to the food) especially in cheese spreads, biscuits and potato flakes (Hudson, 1990). The volatility of BHA makes it useful for addition to packaging materials.
2. Propyl gallate is the n-propyl ester of 3,4,5-trihydroxybenzoic acid, a white crystalline solid. It imparts stability to vegetable oils; provides synergism with BHA and BHT, is heat sensitive and decomposes at its melting point of 148C; has poor "carry through" property; has poor

fat solubility and some water solubility; and can form colored complexes with metal ions, such as iron and copper.

3. BHT (2,6-ditertiarybutyl-4-methylphenol) is a white crystalline solid. It is effective in walnuts, chewing gums and animal fats but not as effective in vegetable oils. Widely used as an industrial antioxidant, BHT is relatively inexpensive.
4. TBH (tertiary-butylhydroquinone), a white-to-tan powder, is the most effective antioxidant for most fats and oils, especially vegetable oils. TBHQ provides fair "carry through" in baking application and excellent "carry through" in frying application, and it has adequate fat and oil solubility.

BHA and BHT, the most widely used artificial antioxidants, have unsurpassed efficacy in various food systems besides their high stability, low cost, and other practical advantages. However, their use in food has been falling off due to suspected action as a promoter of carcinogenesis as well as due to a general public rejection of synthetic food additives (Namiki, 1990). Long term studies have shown that these compounds could produce tumors in animals. Usually additives that have shown to cause cancer in animals are not allowed to be used as food additives, but earlier studies were inconclusive about the effects of BHA and BHT on cancer (Hudson 1990). In a study completed by Yamazake, Yamaguchi, Yamauchi and Kakiuchi (1994), hemorrhage death was observed in rats maintained on BHT. Rats given 1.2 % BHT in the diet for 7 days showed a decrease in coagulation factors and changes of platelet function.

These studies have spurred a gain in popularity of natural antioxidants due to the belief that natural food ingredients are better and safer than synthetics.

EXTENDING SHELF LIFE OF PERISHABLE PRODUCTS BY NATURAL ANTIOXIDANTS.

Various foods contain certain amounts of natural antioxidants among them are tocopherols, ascorbic acids, herbs and enzymes.

-alpha-Tocopherol (vitamin E) as antioxidants

Tocopherols are present in most plants and are responsible for the inherent stability of many vegetable oils. Tocopherols are generally regarded as safe by the FDA. They are isolated from edible vegetable oils and concentrated by molecular distillation (Chrysarn,1994). They work as antioxidants by donation the hydrogen of the hydroxyl group to a fatty peroxide free radical. They show low volatility at high temperatures due to their high molecular weight, and are completely fat-soluble. Alpha-Tocopherol is a powerful antioxidant. It is 250 times more reactive in styrene than the commonly used synthetic antioxidant BHT. In the US, the antioxidant is being used in HDPE mineral water bottles and film for cereal package liners (Smith 1993).

Ascorbic acid (vitamin C) as antioxidants

Found in substantial amounts in some fruits and vegetables, ascorbic acid's principle action is to destroy or eliminate free-radical mediated chain reactions (Chrysarn, 1994). Ascorbic acid has limited uses since it is virtually insoluble in fats and oils, and during processing is often depleted. Ascorbic acid has also been shown to change the flavor of some products. The addition of ascorbic acid is regulated by law and it is not always possible to add enough ascorbic acid to prevent all oxidative reactions (Lehtonen, Aaltonen and Karilainen, 1991). Ascorbic acid has also shown an off flavor in some products.

Plant Extracts as antioxidants

The use of petroleum extracts of sage, nutmeg, rosemary, clove, cardamom, white pepper, black pepper, mace and marjoram as natural antioxidants was reported by (Bassiouny, Hassanien, Razik and El-Kayati, (1989). Also, alkaline and acidic extracts of thyme, marjoram and oregano have been used as natural antioxidants in lard. Some specific applications are crackers, cakes, potato granules, salad dressing, sauces, batter and breading mixes, pickling brines, and snack foods such as nuts, processed meats, seasonings, and pet foods.

A study by Bassiouny et. al (1989) concluded that marjoram, spearmint, peppermint, and basil used as antioxidants in soda crackers:

1. Have an excellent antioxidant effect on the soda cracker compared with the effect of BHA at .01, .02 and .03%.
2. Addition of either BHA or purified ether extracts of all plant materials prevents the accumulation of peroxides after decomposition. This behavior indicates that the presence of synthetic or natural antioxidants slowed down the rate of peroxide formation. In all cases, peroxide values after 135 days of all samples that contained synthetic or natural antioxidants at any given concentration were much lower than that of the samples with no antioxidants.
3. Addition of all samples of plant materials powder at .5% gave an antioxidant effect on the soda cracker biscuit, compared with samples without antioxidant.

4. A new type of biscuit could be produced by adding .5% marjoram powder. This additive improved the taste and odor and extended the shelf life of the product.

Enzyme systems as antioxidants

Researchers have suggested using enzyme systems for removing oxygen from packaged foods. The factors necessary to make enzyme systems workable have not been determined. These factors include:

1. Combinations of enzymes for desired effect,
2. Enzyme levels necessary to ensure creating the desired conditions and,
3. Necessary reactants to cause enzymes to react to products.

Recently, significant production break-throughs have enabled the production of high yields of purified, highly active glucose oxidase that contains little or no contamination with the enzyme catalase. This has made it possible to develop complex enzyme compositions that solve the forgoing difficulties (Lehtonen et al., 1991).

GLUCOSE OXIDASE AS AN ANTIOXIDANT

Discovery of Glucose Oxidase Catalase (GO/CAT)

Glucose oxidase was discovered in 1928 by Muller in *Aspergillus niger and Penicillium glacum*. The enzyme catalyzes the oxidation of D-glucose to -D-gluconolactone in the presence of molecular oxygen (Whitaker, 1972). The enzyme has a molecular weight of 150,000, contains two moles FAD per mole enzyme and has an isoelectric point near pH 4.2.

Current applications of glucose oxidase

Nonenzymatic browning discoloration poses a serious problem in many potato products such as granules, flakes, chips, French fries and hash browns. The problem has become crucial since the use of sulfites as an anti-browning agent has been curtailed or prohibited because of its potential health hazard (Low, Jiang, Ooraikul, Dokhani and Palcic, 1989, p. 118). Low et. al (1989) concluded that the enzyme could be successfully used as a processing aid for potato products to decrease the glucose content and, consequently, the color problems caused by nonenzymatic browning.

Glucose oxidase is used in eggs to remove glucose. Since eggs have a glucose content of approximately .5%, browning and developing off-flavors may occur if the glucose is not removed. This is especially true in the manufacture of dried eggs (Amano International Enzyme Co., 1987, p. 41)

Invention of glucose oxidase catalase (GO/CAT)

In 1991 Lehtonen, Aaltonen and Karilainen received a patent for a composition of GO/CAT enzyme preparation to extend the shelf life of foodstuffs. The method can be used both in normal and modified atmosphere packages. These researchers presented an enzymatic method to preserve foodstuffs where by carefully controlled levels of glucose oxidase and catalase that were added to increase shelf life of food products and also to introduce a catalase to eliminate residual peroxide created by the reaction of glucose oxidase with oxygen.

Proposed applications of glucose oxidase catalase

The use of GO/CAT has been proposed as an on-board preservation system for seafood. When used as a dip, incorporated in ice or immobilized on an algin blanket, glucose oxidase-catalase extended by 67% the shelf life of whole and filleted winter flounder. GO/CAT as an immersion system for the on-board preservation of shrimp had significant preservative effects (Kantt and Torres, 1993).

The enzyme has also been effective in reducing the dissolved oxygen in model salad dressings. Mistry and Min (1992) observed a 92% reduction of dissolved oxygen with .5% GO/CAT during five days storage.

The use of glucose oxidase as an antioxidant is based on the known reaction in which glucose oxidase catalyzes the reaction between glucose and oxygen. In the reaction D-gluconic acid and hydrogen peroxide are produced according the equation: **D-glucose+O_2glucose *oxidase*D = gluconic acid-H_2O_2.**

The reaction continues until either glucose or oxygen is consumed. Hydrogen peroxide is decomposed by catalase into water and oxygen: $\mathbf{H_2O_2}$ **catalase =** $\mathbf{H_2O+1/2O_2}$ (Lehtonen et al., 1991).

Benefits of GO/CAT as antioxidant

Researchers are interested in testing GO/CAT as an antioxidant in oil-containing foods because:

1. The method is natural and non-toxic (Samoszuk, Ehrlich and Ramzi, 1993)
2. Fast reaction time
3. High activity at low concentrations
4. The enzymes can be inactivated by pasteurization after the reaction has continued to the desired extent.

HYPOTHESIS

If Glucose Oxidase Catalase is placed in 8 ounce jars of prepared mayonnaise, no decrease of headspace oxygen from day 1 to day 10 (day 10 = day 1) will be seen.

MATERIALS AND METHODS

The following materials and procedures were followed for the purpose of the study.

Materials

Materials used for the mayonnaise were white vinegar, salt, egg yolk, sugar, and vegetable oil. OxyGO™ M 1500 glucose oxidase was donated from Genencor International®

(Rochester, NY). The mayonnaise was stored in 15 8-oz. Mason jars.

Preparation of mayonnaise

The mayonnaise consisted of 14% vinegar, 2.5% salt, 6% egg yolk, 7% sugar, 70% vegetable oil, 1% water and 0.05% glucose oxidase. Vinegar, water, glucose oxidase, salt and sugar were mixed on low speed in a blender until solids were dissolved. The egg yolks were slowly added with agitation on low, to avoid too much foaming. Once the yolks were blended, the vegetable oil was slowly added until volume began to rise. As the emulsion continued to get whiter, the oil was added and blender speed was increased. Once the oil addition was complete, the blender was increased to maximum speed and the product was mixed for 15 seconds.

Preparation of mason jars

To assure an airtight jar and allow for removal of gas from the headspace for testing, holes were drilled in the lids of the jars and a septum was placed over the hole and sealed with epoxy. Each jar was filled to 6 oz., closed tightly and sealed with epoxy. The jars were than stored at 65°F.

Depletion of headspace oxygen

A .10 ml sample of headspace gas was taken from each of the 15 jars each day for 10 days with a Pressure Lok 1.0 ml gas syringe, and measured with a Hewlett Packard 5890A Gas chromatograph equipped with a Hewlett Packard 3392A integrator. The chromatograph was set up to separate O_2 from the sample gases.

RESULTS

TABLE 2 : % OF OXYGEN IN HEADSPACE OF MASON JARS

	Day #									
Jar #	1	2	3	4	5	6	7	8	9	10
1	17.69	17.85	17.58	16.59	13.74	12.02	10.51	10.66	9.96	8.56
2	19.01	21.1	18.33	15.68	15.13	12.32	10.95	10.63	9.11	7.38
3	20.33	16.27	16.49	16.8	14.77	13.79	11.29	11.37	9.74	8.03
4	21.32	20.03	19.66	15.24	13.96	11.83	10.84	10.68	10.18	6.06
5	18.42	16.96	16.38	15.43	14.68	11.71	11.53	10.24	10.67	7.14
6	19.18	20.1	15.97	14.38	14.3	10.65	11.85	10.45	9.37	5.07
7	20.73	18.03	17.33	17.98	15.01	11.96	12.42	11.78	11.43	6.7
8	21.49	17.35	17.71	17.36	16.52	12.16	12.82	12.25	11.36	6.62
9	20.15	16.55	17.03	14.56	14.23	13.87	13.27	11.67	11.29	6.95
10	20.7	16.8	16.94	15.95	16.46	14.05	11.91	11.35	11.19	10.95
11	17.99	16.17	17.98	15.78	15.5	14.28	12.22	11.98	10.84	10.12
12	18.59	17.62	16.23	15.63	15.77	10.31	12.32	11.8	10.38	10.37
13	20.86	17.1	18.59	16.84	15.87	12.65	12.25	12.21	10.43	8.45
14	19.51	15.91	16.78	14.45	14.15	10.77	12.91	10.04	13.99	8.4
15	19.04	16.57	17.17	15.51	14.79	14.61	14.25	13.17	12.29	8.46

TABLE 3: % REDUCTION OF HEADSPACE IN MAYONNAISE JARS

Jar #	Day 1	Day 10	Difference	% Reduction
1	17.69	8.56	9.13	51.61
2	19.01	7.38	11.62	61.13
3	20.33	8.03	12.3	60.5
4	21.32	6.06	15.27	71.62
5	18.42	7.14	11.28	61.24
6	19.18	5.07	14.11	73.57
7	20.73	6.7	14.03	67.68
8	21.49	6.62	14.87	69.19
9	20.15	6.95	13.2	65.51
10	20.7	10.95	9.75	47.1
11	17.99	10.12	7.87	43.75
12	18.59	10.37	8.22	44.22
13	20.86	8.45	12.41	59.5
14	19.51	8.4	11.12	57
15	19.04	8.46	10.58	55.57
AVERAGE	19.67	7.95	11.72	59.28

Statistical analysis

Using the data obtained from the test, a paired sample t-test (Microsoft Excel 5.0) was completed at alpha =.05 to determine the following hypothesis:

If Glucose Oxidase Catalase is placed in 8 ounce jars of prepared mayonnaise no decrease of headspace oxygen from day 1 to day 10 (day 10 = day 1) will be seen.

Table 4 shows the results of the t-test. The results indicate that there is a significant difference between the mean of day 1 compared with the mean of day 10. This is shown by the t-statistic and the t-critical value. The t-statistic is larger than the t-critical that indicates that the two means compared are significantly different. The probability of the two means being equal is indicated by P(T<=t) which is 0. Therefore Glucose Oxidase Catalase did in fact reduce headspace oxygen in an airtight package.

TABLE 4: TWO SAMPLE T-TEST TO DETERMINE STATISTICAL SIGNIFICANCE OF GLUCOSE OXIDASE ON HEADSPACE OXYGEN

	Day 1	Day 10
Mean	19.67	7.95
Variance	1.49	2.7
Observations	15	15
Pearson Correlation	-0.31	
Hypothesized Mean Difference	0	
df	14	
***t Stat**	**19.46**	
P(T<=t)	0	
***t Critical**	**2.14**	

since t stat is larger than t critical there is a significant difference between day 1 and day 10 indicating that the glucose oxidase catalase reduced head space oxygen significantly.

CONCLUSIONS AND APPLICATIONS

The objective of this study was to determine the effect of Glucose Oxidase in reducing headspace oxygen in an airtight package. Glucose Oxidase (0.05%) was included in the standard recipe for mayonnaise. A 0.10 ml of headspace gas was removed daily for 10 days from 15 jars of mayonnaise. A paired t-test ($t=19.46$, $df=14$, $p<0.00$) rejected the hypothesis that the addition of Glucose Oxidase would not remove the headspace O2.

This study has many applications for the food packaging industry.

1. Little is known concerning the ingestion of synthetic preservatives on human subjects. Although studies on animal subjects indicate that these preservatives are harmful, the studies have not been replicated on humans. However, consumers assume that the preservatives are harmful and are seeking alternative products.
2. GO/CAT may extend the shelf life by continuing the reactions with oxygen in mayonnaise after the consumer opens the package and stores it in the refrigerator.
3. GO/CAT may reduce the proliferation of pathogenic bacterial in foods prepared with mayonnaise resulting in reduced risk of food borne illnesses.

4. Base on the positive results, the study could be duplicated substituting other high fat products such as, other salad dressings, margarine, animal fat products, oil or other foods susceptible to oxygenation.

Recommendations for further study

The following are Research or testing oppurtunities for another Masters student:

1. Continue measuring headspace reduction for an extended time to see if Glucose Oxidase will eliminate all headspace oxygen.
2. The ability of Glucose Oxidase to continue to reduce headspace oxygen after the mayonnaise jar has been opened.

APPENDIX

Individual Graphs -- Headspace Reduction of Mayonnaise Jars

FIGURE 1: REDUCTION OF HEADSPACE OXYGEN JAR #1

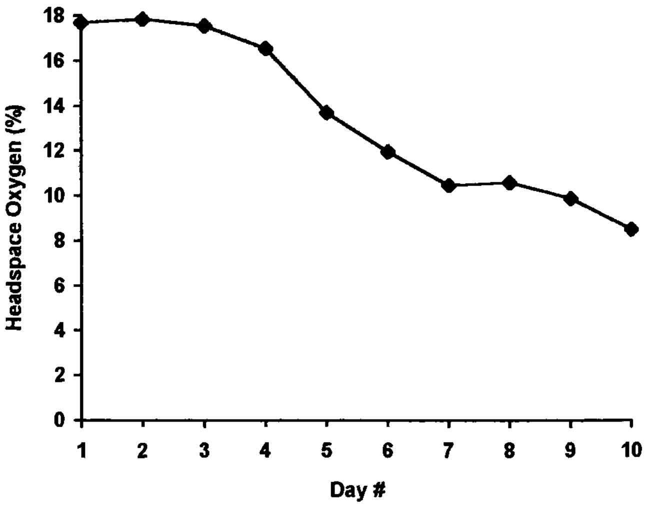


FIGURE 2: REDUCTION OF HEADSPACE OXYGEN JAR #2

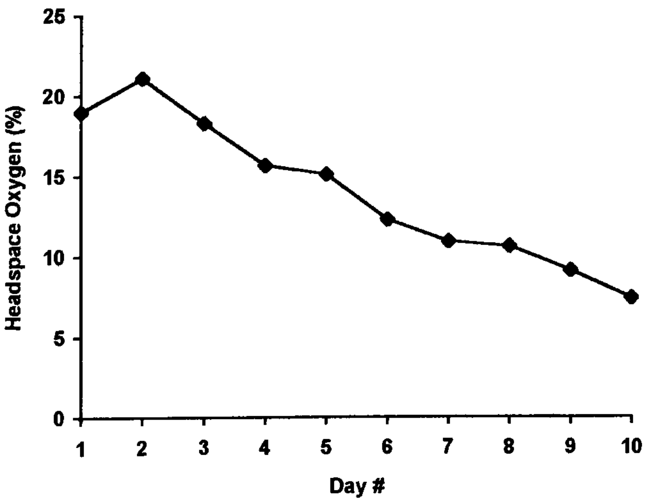


FIGURE 3: REDUCTION OF HEADSPACE OXYGEN
JAR #3

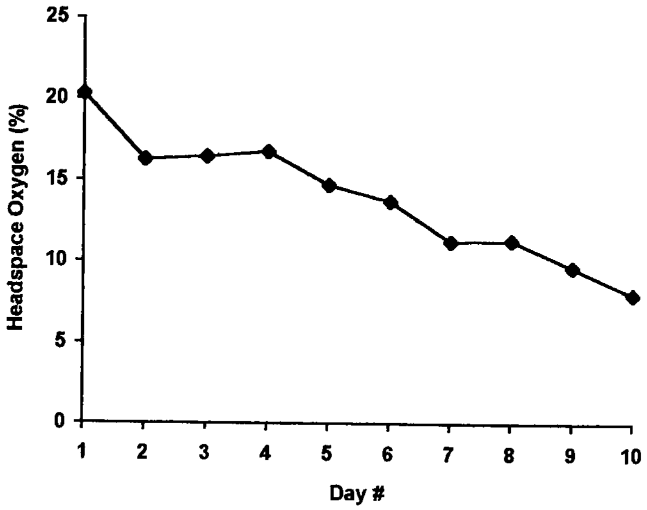


FIGURE 4: REDUCTION OF HEADSPACE OXYGEN
JAR #4

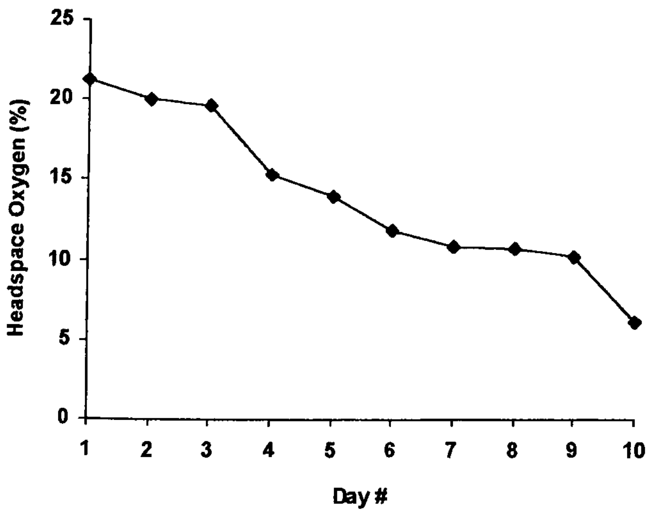


FIGURE 5: REDUCTION OF HEADSPACE OXYGEN
JAR #5

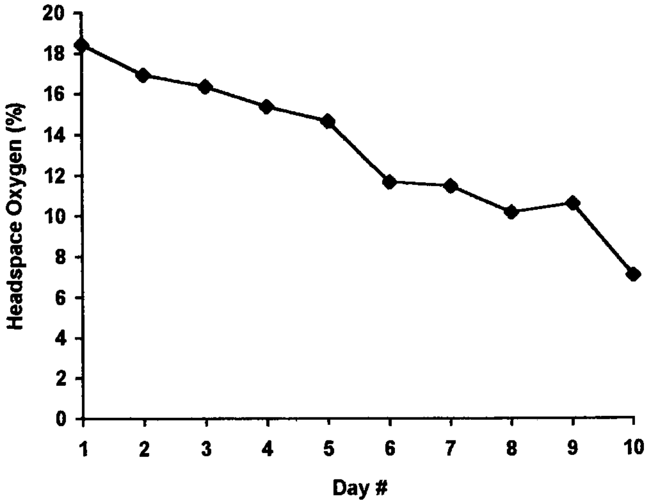


FIGURE 6: REDUCTION OF HEADSPACE OXYGEN
JAR #6

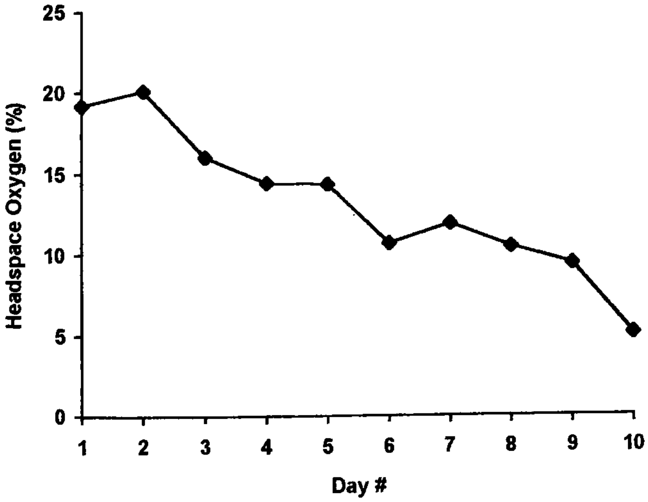


FIGURE 7: REDUCTION OF HEADSPACE OXYGEN
JAR #7

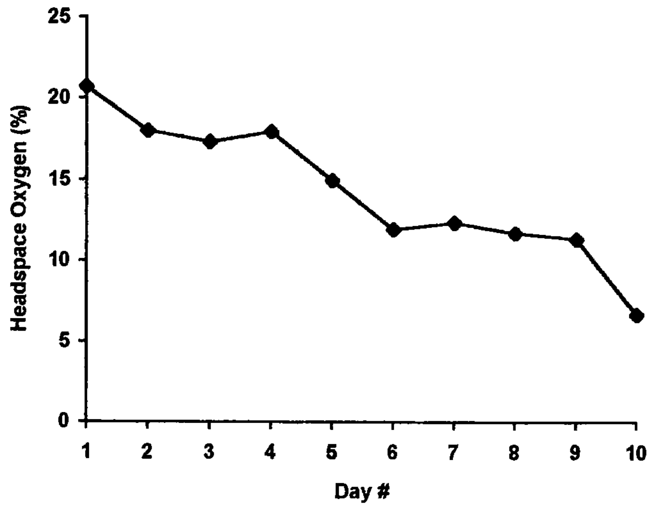


FIGURE 8: REDUCTION OF HEADSPACE OXYGEN
JAR #8

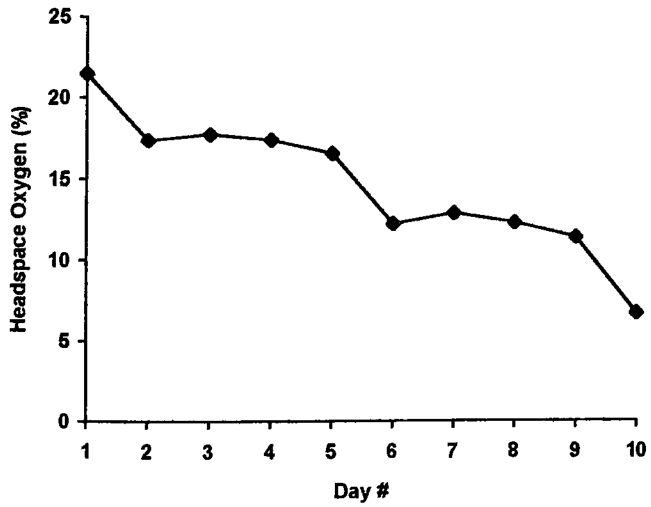


FIGURE 9: REDUCTION OF HEADSPACE OXYGEN
JAR #9

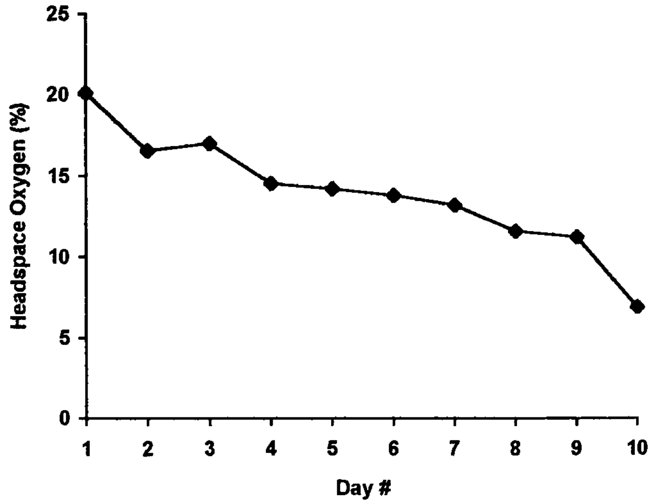


FIGURE 10: REDUCTION OF HEADSPACE OXYGEN
JAR #10

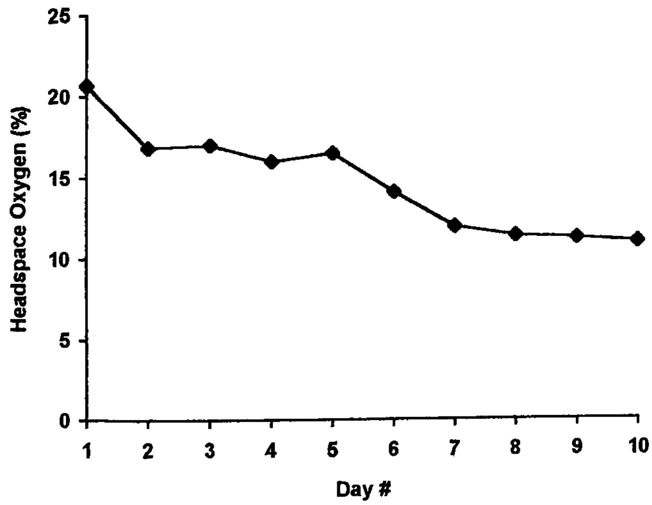


FIGURE 11: REDUCTION OF HEADSPACE OXYGEN
JAR #11

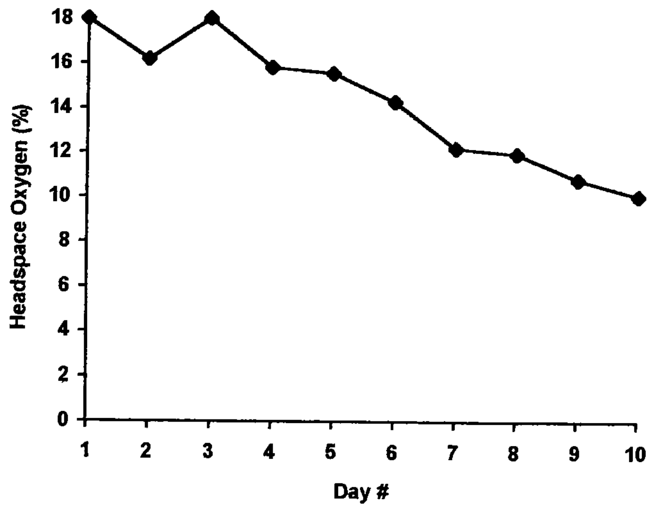


FIGURE 12: REDUCTION OF HEADSPACE OXYGEN
JAR #12

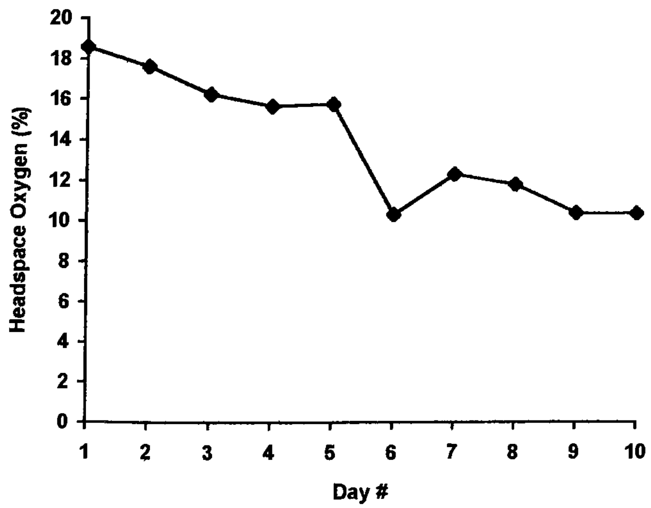


FIGURE 13: REDUCTION OF HEADSPACE OXYGEN
JAR #13

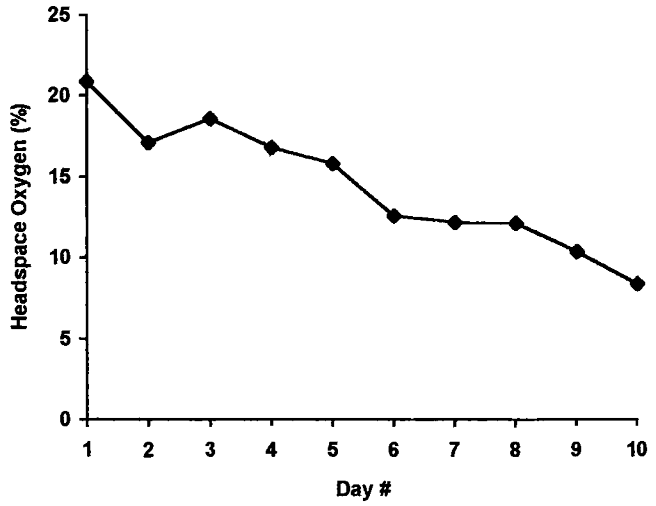


FIGURE 14: REDUCTION OF HEADSPACE OXYGEN
JAR #14

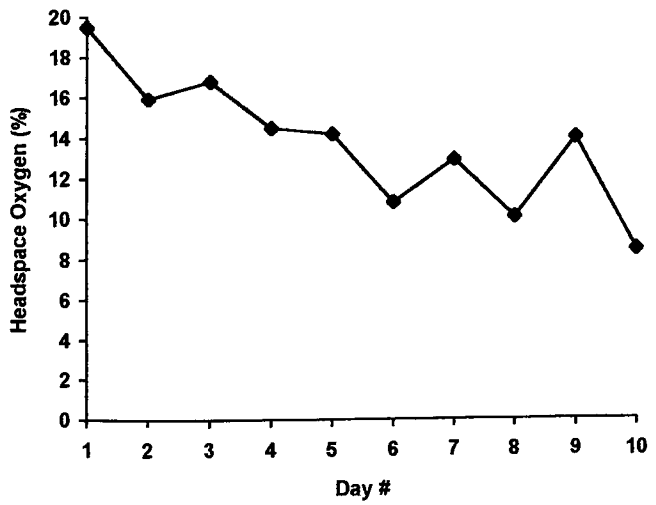
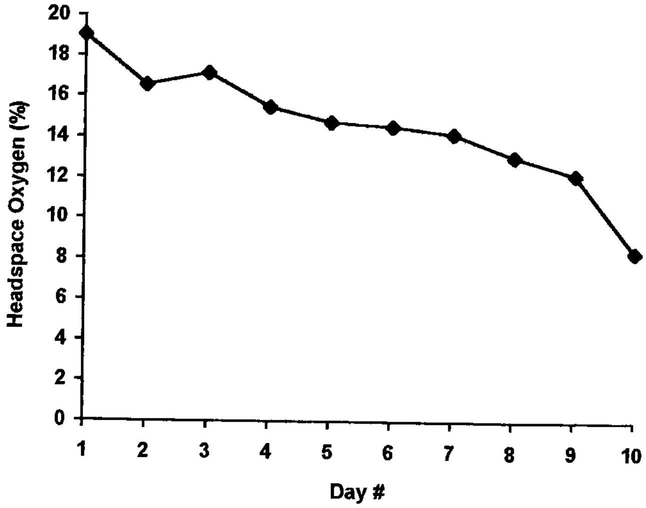


FIGURE 15: REDUCTION OF HEADSPACE OXYGEN
JAR #15



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